impaired balance control. This study aimed to determine whether people with hip chondropathy demonstrate impaired balance ability during a dynamic single-leg squat with eyes open (SquatEO) and single-leg standing task with eyes closed (StandEC), relative to controls. A secondary aim was to explore whether hip range of motion (ROM) and hip muscle strength were correlated to balance measures in the hip chondropathy group.

Methods: 63 adults (36 female, mean [SD] age: 37.6 [11.6] years) with hip chondropathy (diagnosed arthroscopically using the Outerbridge classification for chondral damage in the previous 12-24 months) and 60 healthy controls (41 female, mean [SD] age: 35.7 [9.7] years) performed two single-leg balance tasks: SquatEO and StandEC while standing on a Nintendo WiiTM balance board. Tests were performed barefoot, on the surgical leg of hip chondropathy participants and dominant leg of controls. Centre of pressure (CoP) total path velocity, range and standard deviation (SD) of CoP movement in the mediolateral (ML range & MLSD) and anterior-posterior direction (AP range & APSD) were extracted. Hip ROM and muscle strength were measured with an inclinometer and hand-held dynamometer. Data were analysed using a one-way ANOVA and stepwise multiple regression. The alpha level was set to 0.05.

Results: During the SquatEO, greater CoP ML range ($P=0.001$) and APSD ($P=0.030$) was observed in those with hip chondropathy compared to healthy controls. No significant between-group differences were observed for any of the balance measures during the StandEC ($P>0.05$). Multiple regression analyses identified hip external rotation ROM as being significantly associated with ML range during the SquatEO ($P=0.005$). When performing the SquatEO, hip external rotation ROM accounted for 11% of the variance in CoP ML range. No hip measurements were correlated to APSD during the SquatEO.

Conclusions: Dynamic single-leg balance squat performance is reduced in young adults with hip chondropathy compared to healthy adults, but single-leg standing balance is not. CoP ML range and APSD were significantly greater in those with hip chondropathy relative to controls, which can be interpreted as reduced control of dynamic movements. This study suggests early signs of hip joint degeneration can impair postural control mechanisms, particularly during dynamic tasks incorporating an internal perturbation. Those with greater hip joint external rotation motion appear to have worse single-leg squat balance performance. Further investigation into balance deficits associated with hip disease is necessary to establish early-identification strategies and a more tailored-approach to rehabilitation. Greater understanding of mechanical, muscular and neurophysiological processes for balance control in adults with hip chondropathy, early- and advanced hip OA is essential.

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DISABILITY AND NOT OSTEOARTHRITIS PREDICTS CARDIOVASCULAR DISEASE; A PROSPECTIVE POPULATION-BASED COHORT STUDY

T.A. Hoeven, M.J. Leening, P.J. Bindels, M. Castano-Betancourt, J.B. van Meurs, O.H. Franco, M. Kavousi, A. Hofman, A.M. Ikram, J.C. Witteman, S.M. Bierma-Zeinstra. *Erasmus MC, Rotterdam, Netherlands*

Purpose: Osteoarthritis (OA) is associated with many comorbid conditions, and a recent interest in linking osteoarthritis to cardiometabolic disorders has emerged. Several cross-sectional and disease-specific mortality studies suggest an association between OA and cardiovascular disease (CVD). This association might arise from different potential mechanisms. Possibly, they are concurrent diseases that share aetiological features and risk factors. Otherwise, atherosclerotic disease might play an initiating role by causing microcirculatory disturbances in the synovial membrane and subchondral bone that contribute to the cartilage destruction and pathophysiological process of OA. However, we found no indications for this latter pathway in a large-scale longitudinal study. Finally, OA might lead to a sedentary lifestyle, disability and therefore an increased risk of CVD. For the development of treatment strategies, the mechanism behind the relation between OA and CVD is relevant. When OA patients are at risk for CVD, preventive measures in clinical practice are needed. We investigated whether OA is indeed an additional risk factor for the development of CVD, and - since OA and disability are closely related - examined the role of disability in the association between OA and CVD.

Methods: Our study was embedded in The Rotterdam Study, a prospective population-based cohort study in a suburb in the city of Rotterdam, the Netherlands. At baseline 4,648 persons aged $\geq$55 and free of CVD were classified on the basis of radiographs of the knee, hip, or hand and symptoms of OA into those with and those without OA. For the assessment of disability the Stanford Health Assessment Questionnaire was used. We categorized persons as having any disability versus none, independent of the presence of OA. Hazard ratios adjusted for cardiovascular risk factors for developing CVD (a composite of coronary heart disease and stroke) were calculated.

Results: During a median follow-up time of 14.4 years 1,230 cardiovascular events (myocardial infarction, surgical or percutaneous coronary revascularisation, coronary mortality and stroke) occurred, of which 101 in the clinical knee OA group. Presence of radiographic knee OA, defined as a Kellgren&Lawrence score $\geq$2 in at least one joint, was not related to future CVD (HR 0.99, 95%CI 0.86 to 1.15), neither was presence of clinical knee OA, defined as a Kellgren&Lawrence score $\geq$2 and complaints in the same joint during the last month (HR 1.09, 95%CI 0.88 to 1.34) (see table 1; model 1 was adjusted for age and sex, model 2 was adjusted for age, sex, body mass index, diabetes, hypertension, total cholesterol /HDL cholesterol ratio, and current smoking). Results for hand OA or hip OA were not substantially different from knee OA. However, persons with increasing disability were more likely to suffer from a cardiovascular event compared with non-disabled persons (HR 1.26, 95%CI 1.12 to 1.42); this was independent of the presence of OA.

Conclusions: In this population-based study, we found that the presence of OA was not related to risk of CVD. On the other hand, disability was a predictor of future CVD independent of the presence of OA. In clinical practice, having OA is not another red flag to start CVD prevention apart from established cardiovascular risk factors, such as hypertension and diabetes. Disability, however, should be prevented, also in the absence of OA. The close relation between disability and OA may explain earlier findings of a relation between OA and risk of CVD.

Knee osteoarthritis and risk of incident cardiovascular disease.

		Hazard ratio (95%CI)	P-value
		Total CVD (n=1230)	
Radiographic OA	Model1	1.00 (0.87 to 1.15)	0.96
	Model2	0.99 (0.86 to 1.15)	0.92
Clinical OA	Model1	1.08 (0.88 to 1.33)	0.45
	Model2	1.09 (0.88 to 1.34)	0.43
Self-reported OA	Model1	1.08 (0.93 to 1.24)	0.32
	Model2	1.09 (0.94 to 1.26)	0.26

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P21 REGULATES MMP-13 EXPRESSION THROUGH STAT3 SIGNALING IN CHONDROCYTES

S. Hayashi, T. Nishiyama, S. Hashimoto, T. Fujishiro, N. Kanzaki, K. Iwasa, S. Sakata, N. Chinzei, R. Kuroda, M. Kurosaka. *Kobe Univ., Kobe, Japan*

Purpose: Osteoathritis (OA) is a multifactorial disease, and biomechanical stress is a major contributor to OA pathology. However, the underlying mechanisms remain unclear. Recent data have suggested that cell cycle-related proteins play a role in OA pathology. The cyclin-dependent kinase inhibitor p21 was initially identified as a potent inhibitor of cell cycle progression. Recently, however, it has been proposed that p21 is a regulator of regulates transcription factor activity. In this study, we evaluated the role of p21 in the control of gene expression during the response to cyclic tensile strain, an experimental model of biomechanical stress.

Methods: Normal human knee chondrocytes (Cambrex, Charles City, IA) were cultured and used in this study. Chondrocytes were treated with p21-specific siRNA, and cyclic tensile strain was introduced in the presence or absence of a STAT3-specific inhibitor. Eight-week-old male C57BL/6J mice were used in this experiment. Destabilization of the medial meniscus (DMM) was induced in the right knee joint and sham surgery was performed in the left knee joint. Mice were sacrificed at 12 weeks after DMM surgery and subjected to extraction of mRNA.

Results: The expression of *MMP-13* mRNA increased in response to 5% cyclic tensile strain following transfection with p21 siRNA, whereas the

expression of *aggrecan* was decreased (Figure 1). Phospho-STAT3 and MMP-13 mRNA levels were increased by downregulation of p21, and this was reversed by treatment with a STAT3 inhibitor (Figure 2a, b). Downregulation of p21 also decreased proteoglycan synthesis (Figure 2c). In a mouse OA model and in human primary chondrocytes, we found that p21 mRNA levels were reduced, whereas SDF-1 and CXCR4 expression was increased (Figure 3). In OA primary chondrocytes, expression of p21 is decreased and phosphorylation of STAT3 significantly increased (data not shown).

Conclusions: Our results suggest that p21 in chondrocytes functions to maintain matrix synthesis by regulation of aggrecan and MMP-13 expression via STAT3 phosphorylation. Because p21 levels are reduced in OA chondrocytes, our data imply that stabilization of p21 may be a therapeutic strategy for OA treatment.

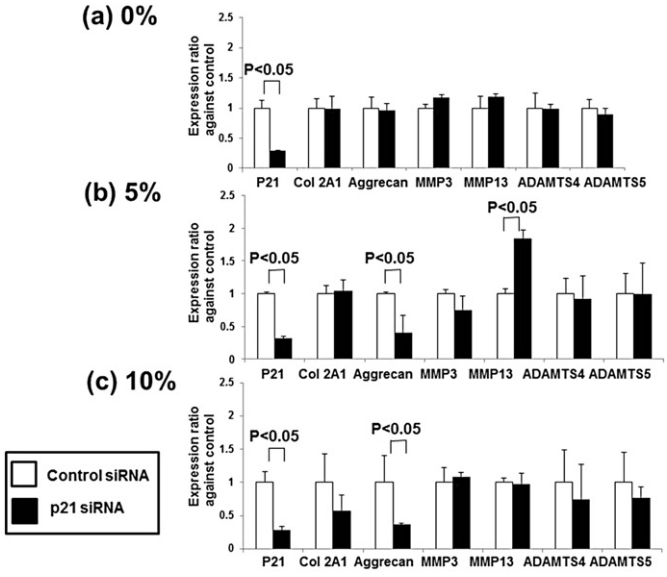

Fig 1. Knockdown efficiency of p21 siRNA transfection to human normal Chondrocytes in response to 0%, 5%, and 10% cyclic tensile strain. The concentration of p21 siRNA is 150 nM. Expression levels of *p21, COL2A1, ACAN, MMP-3, MMP-13, ADAMTS-4*, and *ADAMTS-5* mRNA were quantified by real-time PCR.

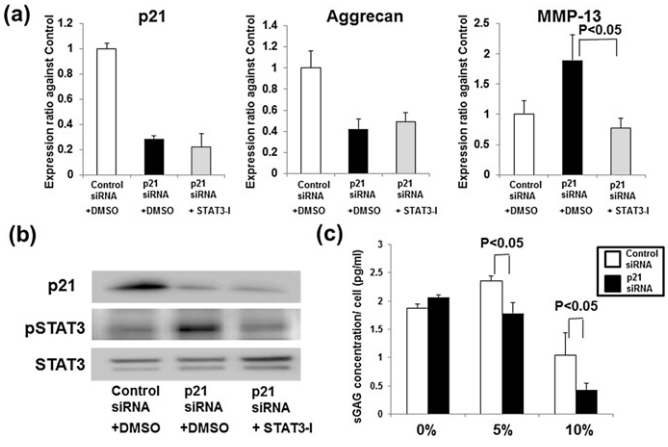

Fig 2. The effect of a STAT3-specific inhibitor on p21-regulated ACAN and MMP-13 expression. Chondrocytes were transfected with p21 siRNA or nonspecific control siRNA for 12 h, and 0%, 5%, or 10% cyclic stretch stress was introduced for 24 h in the presence of 50 μM DMSO or STAT3 specific inhibitor. (a) Expression levels of *p21, ACAN*, and *MMP-13* mRNAs were quantified by real-time PCR. (b) p21 and phospho-STAT3 were analyzed by western blotting. (c) Effect of p21 knockdown on sulfated glycosaminoglycan

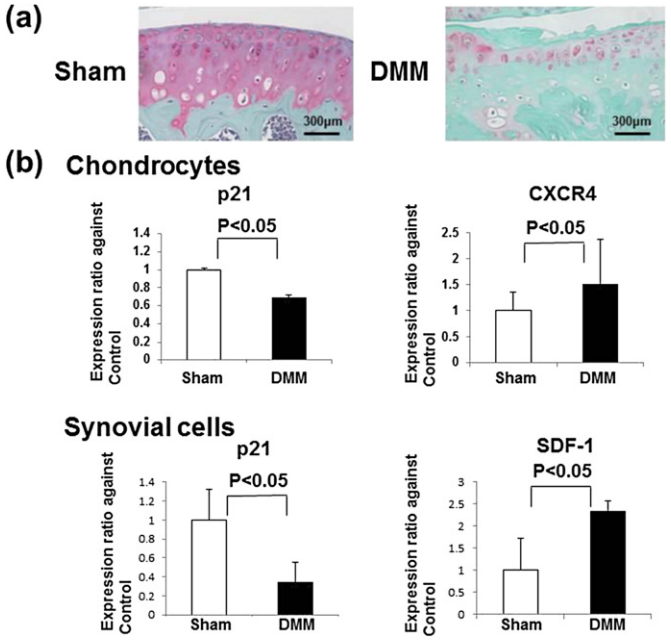

Fig 3. For in vivo experiments, cartilage tissue samples were collected from right knee (DMM surgery) and left knee (sham control). (a) The sections were stained with Safranin O and Fast Green. (b) Expression levels of *p21, CXCR4,* and *SDF-1* mRNAs were quantified by real-time PCR. Expression ratios of p21, CXCR4 and SDF-1 are shown. Columns represent mean ± SD ratios of DMM/control.

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NO INTERVERTEBRAL DISC WITH A FLAT TIRE, NOVEL HUMAN ANNULUS FIBROSIS CELL MODELS

G.G. van den Akker[†,‡], T.J. Welting[‡], D.A. Surtel[‡], A. Cremers[‡], J.W. Voncken[†], L.W. van Rhijn[‡]. [†]*Maastricht Univ., Dept of Molecular Genetics, Maastricht, Netherlands;* [‡]*Maastricht Univ., Dept of Orthopaedic Surgery, Maastricht, Netherlands*

Purpose: The integrity of the annulus fibrosis (AF) tissue in the intervertebral disc is crucial to avoid herniation and low back pain. In relation to disc degeneration, the cell biology and pathology of the AF is little studied, compared to the nucleus pulposus (NP). To provide a functional NP microenvironment and support the overall spinal compartmentalization, however, the integrity of the fibrous AF is pivotal. As in vitro models to study AF cells are not available, we set out to generate clonal cell lines derived from the human annulus fibrosis.

Methods: Non-degenerated human disc material was obtained from young adolescent donors as surgical surplus material. We immortalized early passage monolayer cultures by retroviral expression of the SV40LTAg and hTERT genes. We generated 33 clonal cell lines from the immortal AF cell pool using a limiting dilution method and mRNA and protein markers were measured that in vivo discriminate between the nucleus pulposus and annulus fibrosis to provide a detailed characterization of the AF phenotype.

Results: AF clones yielded a predominant subtype that morphologically differed from primary and immortalized cell pools. Isolated AF clones showed a strong tendency to migrate and become organized in patterned alignments (Figure 1A). AF clones synthesized mainly Collagen type I (COL1A1) and little COL2A1 protein (Figure 1 B). Furthermore AF clones showed high gene expression of FBLN1, COL1A1 and COMP, whereas expression of the NP marker genes CA12, FOXF1 and KRT19 in comparison with NP clones was low. Chondrogenic differentiation induced down regulation of COL1A1 expression and cells spontaneously formed compact aggregates within 5-7 days (Figure 1B). In contrast, nucleus pulposus-derived clones upregulated SOX9 and COL2A1 expression and formed less dense aggregates. Cell surface marker expression was determined for CD24, CD44 and multiple MSC markers in AF and nucleus pulposus clonal cell lines. AF clones were positive for CD44, CD90 and negative for CD24, CD73 and CD105; nucleus pulposus cell clones were found positive for all of the CD